

Original Research Article

Amino Acid Dysmetabolism in Type 2 Diabetes Mellitus: Integrated Analysis of Urinary Chromatography and Serum Amino Acid Profiles

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ABSTRACT

Background: Type 2 diabetes mellitus (T2DM) represents a complex metabolic disorder associated with significant disruptions in glucose and amino acid metabolism. Growing evidence indicates that altered plasma amino acid profiles and increased urinary amino acid excretion (aminoacidurias) are defining metabolic features of T2DM, reflecting insulin resistance, enhanced gluconeogenesis, and impaired protein metabolism.

Materials and methods: In this case-control study, 115 patients with type 2 diabetes mellitus and 115 age- and sex-matched healthy controls were enrolled. Urinary aminoacidurias were screened using paper chromatography, and serum amino acid profiles were analysed using GC-MS. Differences in amino acid patterns and their correlations with disease severity and metabolic parameters were assessed.

Results: The study offers new insights into altered amino acid metabolism in type 2 diabetes and identifies potential biomarkers for diagnosis and disease monitoring. These findings suggest future therapeutic avenues and improve understanding of the metabolic complexity of type 2 diabetes.

Conclusion: These findings emphasise the utility of amino acid profiling as a diagnostic and therapeutic approach in type 2 diabetes mellitus management.

Keywords: Amino acids; aminoacidurias; Gas chromatography-tandem mass spectrometry; Paper chromatography; Type 2 diabetes mellitus

INTRODUCTION

Type 2 diabetes mellitus (T2DM) has emerged as a major global public health challenge, currently affecting more than 460 million people worldwide. This burden is projected to increase to approximately 700 million by 2030, with a disproportionate impact on low- and middle-income countries.¹ The rising prevalence of T2DM is driven by multiple factors, including sedentary lifestyles, unhealthy dietary habits, obesity, and genetic susceptibility. In addition

to its growing incidence, T2DM substantially increases the risk of serious complications such as cardiovascular disease, kidney failure, and other debilitating conditions. As the global burden of diabetes continues to escalate, a deeper understanding of the complex pathophysiology of T2DM, along with the identification of effective prevention and therapeutic strategies, has become critically important.² Specific amino acids play a vital role in maintaining glucose homeostasis and regulating

insulin secretion from pancreatic β -cells. Diabetes-associated alterations in amino acid metabolism—including disruptions in the synthesis, transport, and utilisation of specific amino acids—are closely linked to insulin resistance and the progression of T2DM. A key manifestation of these metabolic disturbances is aminoaciduria, defined as the excessive urinary excretion of amino acids. This phenomenon highlights the intricate interplay between glucose and amino acid homeostasis, which may further aggravate insulin resistance and glucose intolerance.^{3,4} In T2DM, diabetes-induced alterations in renal function, including impaired tubular reabsorption and changes in amino acid transporter activity, contribute to the development of aminoaciduria. These abnormalities disrupt protein synthesis and tissue repair processes, thereby exacerbating the metabolic dysfunction associated with the disease. Elucidating the metabolic significance of these diabetes-related alterations, particularly in the context of aminoaciduria, may help identify novel therapeutic targets for addressing protein-energy wasting and improving glycaemic control in patients with diabetes.⁵⁻⁷

The primary objectives of this study were: (a) to investigate and compare the prevalence of specific aminoacidurias in patients with T2DM and healthy controls; (b) to analyse and compare serum amino acid profiles between T2DM patients and healthy controls; and (c) to evaluate the association between the degree of aminoaciduria and glycaemic control, as assessed by glycated haemoglobin (HbA1c), in patients with T2DM.

MATERIALS AND METHODS

This cross-sectional study included 115 patients with type 2 diabetes mellitus who were receiving oral antidiabetic medications and had a disease duration of

less than 10 years from the time of diagnosis, as defined by American Diabetes Association (ADA) criteria, along with 115 healthy control subjects. Participants of both genders, aged 25–65 years, were enrolled.

The sample size was calculated using the formula.

$$n = (Z\alpha/2 + Z1-\beta)^2 \times (\sigma_1^2 + \sigma_2^2) / (\delta)^2$$
, based on a 95% confidence interval.

The study was conducted in the Department of Biochemistry and Written informed consent was obtained from all participants prior to enrolment.

Patients with type 1 diabetes mellitus, type 2 diabetes on insulin therapy, urinary tract infections, renal failure, diabetic nephropathy, renal glycosuria, or known inborn errors of metabolism were excluded from the study.

Sample Collection and Biochemical Analysis

Whole Blood and Plasma: Under aseptic conditions, 2 mL of venous blood was collected after an overnight fast into EDTA vacutainers. Glycated haemoglobin (HbA1c) was estimated using the high-performance liquid chromatography (HPLC) method. The samples were subsequently centrifuged to separate plasma, which was used for the estimation of fasting blood glucose by the glucose oxidase–peroxidase (GOD–POD) method on a fully automated integrated biochemistry analyser (AU480 analyzer-Beckman Coulter Inc.)

All serum samples were stored at -80°C until analysis. Serum levels of specific amino acids were quantified using gas chromatography–tandem mass spectrometry (GC–MS/MS) in accordance with the manufacturer's instructions.⁸

Paper Chromatography (PC)

Paper chromatography (PC) is a versatile, inexpensive, and non-invasive analytical technique used to separate and identify amino acids and other

biomolecules based on their affinity for a stationary phase and their mobility in a mobile phase, expressed as the retention factor (Rf). PC was standardized in the central research biochemistry laboratory using standard amino acids.

The solvent system was prepared in a ratio of 12:3:5 (v/v/v) using butanol, acetic acid, and water, respectively. Amino acids were visualized using a 0.1% ninhydrin solution in acetone. All 18 commercially available standard amino acid solutions were applied individually as 4 µL spots onto chromatography paper and allowed to air-dry. The paper was then placed in a chromatography chamber containing the solvent system and allowed to develop until the solvent front migrated 10–15 cm, which typically required 2–4 hours.

After development, the paper was air-dried at room temperature for 30 minutes. A 0.1% ninhydrin solution was uniformly sprayed over the paper to ensure consistent staining of amino acid spots while avoiding excess moisture. The paper was then incubated at room temperature for 30 minutes, followed by heat treatment at 100°C for 2 minutes to allow colour development. The Rf values for each amino acid were calculated using the formula:

$$Rf = \frac{\text{distance travelled by the solute}}{\text{distance travelled by the solvent front}}$$

This chromatogram served as the reference standard for amino acid identification.

An early-morning, first-voided midstream urine sample (5 mL) was collected from both patients with type 2 diabetes mellitus and healthy controls in sterile containers. The samples were centrifuged to remove cellular debris, and the clear supernatant was used for chromatographic analysis. Urine samples were applied as 4 µL spots onto chromatography paper and developed using the same solvent system. Amino

acids in urine samples were identified by comparing their Rf values with those obtained from the standard reference chromatogram.

RESULTS

In the present study, 115 patients with type 2 diabetes mellitus and 115 healthy controls were enrolled in accordance with the predefined inclusion and exclusion criteria. Age-wise distribution revealed that the majority of patients with type 2 diabetes belonged to the 50–60-year age group compared with the control group, as shown in **Table-1**. Gender-wise analysis indicated a higher proportion of male participants than females in both groups, as presented in **Table-2**. Baseline clinical and biochemical parameters are summarized in **Table-3**. Fasting blood glucose, systolic blood pressure, diastolic blood pressure, and HbA1c levels were significantly higher in patients with type 2 diabetes compared with healthy controls ($p < 0.001$). In addition, patients with type 2 diabetes were significantly older than the control group ($p < 0.001$). The overall frequency of specific aminoacidurias was markedly higher in patients with type 2 diabetes than in healthy controls. Aminoacidurias of glutamic acid (58.26% vs. 9.57%), valine (34.78% vs. 4.35%), lysine (16.52% vs. 9.57%), leucine (11.30% vs. 1.74%), alanine (23.48% vs. 2.61%), serine (25.22% vs. 2.61%), tryptophan (10.43% vs. 0.87%), phenylalanine (10.43% vs. 1.72%), glycine (10.43% vs. 2.61%), histidine (20.00% vs. 3.48%), tyrosine (6.09% vs. 0%), proline (10.43% vs. 1.74%), cystine (11.30% vs. 4.35%), and arginine (6.96% vs. 1.74%) were significantly more prevalent in the diabetic group ($p < 0.05$). Aminoacidurias of isoleucine, aspartic acid, threonine, and methionine did not reach statistical significance, although their prevalence was higher among patients

with type 2 diabetes in **Table-4**. Comparison of the mean Rf values between the two groups demonstrated significantly higher Rf values on paper chromatography in patients with type 2 diabetes compared with healthy controls ($p < 0.001$), as shown in **Table-5**.

Table-6 illustrates the correlation between glycaemic control and the degree of aminoaciduria, defined as the number of amino acids detected in urine by paper chromatography. A significant positive correlation was observed between the degree of aminoaciduria and elevated HbA1c levels, indicating a direct association between poor glycaemic control and impaired renal amino acid reabsorption. To our knowledge, this is the first study to establish a relationship between the extent of aminoaciduria and glycaemic control in patients with T2DM.

Plasma concentrations of specific amino acids analysed in serum are presented in **Table-7**. Levels of glutamic acid, valine, lysine, leucine, isoleucine, tyrosine, threonine, and arginine were significantly increased, whereas glycine, alanine, and histidine levels were significantly decreased in patients with type 2 diabetes compared with healthy controls ($p < 0.001$). No statistically significant differences were observed for serine, tryptophan, phenylalanine, aspartic acid, methionine, proline, and cystine ($p > 0.05$). Branched-chain amino acids (BCAAs), including leucine, isoleucine, and valine, are essential amino acids that play a critical role in glucose metabolism and insulin sensitivity.

Table-1: Frequency distribution of age of healthy controls and type 2 diabetic subjects.

Age interval	Controls		Diabetes mellitus	
	<i>n</i> = 115	%	<i>n</i> = 115	%
≤30	60	52.17	10	8.70
31–40	17	14.78	13	11.30
41–50	13	11.30	29	25.22
51–60	25	21.74	63	54.78

Table-2: Frequency distribution of gender of healthy controls and type 2 diabetic subjects.

Gender	Controls		Diabetes mellitus	
	<i>n</i> = 115	%	<i>n</i> = 115	%
Male	89	77.39	68	59.13
Female	26	22.61	47	40.87

DISCUSSION

Aminoaciduria, defined by excessive urinary excretion of amino acids, is a common complication in patients with type 2 diabetes mellitus and reflects impaired renal tubular reabsorption and disrupted metabolic pathways. Increased urinary loss of essential amino acids, particularly branched-chain amino acids (BCAAs), may further aggravate insulin resistance and disturbances in glucose homeostasis. Evidence suggests that aminoaciduria in T2DM is associated with heightened oxidative stress, inflammation, and progressive renal injury.

Table-3: Comparison of baseline parameters between healthy controls and type 2 diabetic subjects using t-test

Variables	Controls	Diabetes mellitus	t-test	p value	Significance
Age	34.77 ± 13.73	49.1 ± 11.13	-8.699	0.00001	Significant
Systolic blood pressure	118.29 ± 8.68	134.6 ± 16.54	-9.364	0.00001	Significant
Diastolic blood pressure	80.82 ± 6.36	87.76 ± 6.02	-8.499	0.00001	Significant
Fasting blood glucose	89.36 ± 19.92	190.8 ± 143.34	-7.520	0.00001	Significant
Serum creatinine	0.71 ± 0.22	0.83 ± 0.69	-1.799	0.07335	Not Significant
HbA1c	5.28 ± 0.29	8.93 ± 2.15	-18.02	0.00001	Significant

Table-4: Comparing specific aminoacidurias between healthy controls and type 2 diabetic subjects' group by using normal Z-test.

Amino acids	Controls (%)	Diabetes mellitus (%)	Z-test	p value	Significance
Glutamic acid	9.57	58.26	-9.095	0.00001	Significant
Valine	4.35	34.78	-6.299	0.00001	
Lysine	6.09	16.52	-2.533	0.00565	
Leucine	1.74	11.30	-2.994	0.00138	
Alanine	2.61	23.48	-4.942	0.00001	
Isoleucine	0.87	1.74	-0.582	0.28042	Not Significant
Serine	2.61	25.22	-5.241	0.00001	Significant
Tryptophan	0.87	10.43	-3.211	0.00066	
Phenylalanine	1.74	10.43	-2.805	0.00252	
Glycine	2.61	10.43	-2.434	0.00746	
Histidine	3.48	20.00	-4.027	0.00003	
Tyrosine	0.00	6.09	-2.730	0.00317	Not Significant
Aspartic acid	2.61	6.09	-1.298	0.09713	
Threonine	0.00	1.74	-1.427	0.07684	
Methionine	1.74	4.35	-1.155	0.12407	Significant
Proline	1.74	10.43	-2.805	0.00252	
Cystine	4.35	11.30	-1.981	0.02381	
Arginine	1.74	6.96	-1.956	0.02523	

Table-5: Comparing the mean Rf values of paper chromatography in healthy controls and type 2 diabetic subjects

Amino acids	Controls	Diabetes mellitus	Z-test	p value
Rf value	0.142 ± 0.066	0.312 ± 0.062	-3.095	0.00001

Table-6: Correlation between glycaemic control and degree of aminoacidurias in type 2 diabetic subjects.

HbA1c level (%)	Glycaemic control grading	Degree of amino aciduria	R-value	T-value	P value
< 6.5	Excellent control	0–2	0.12	1.23	0.22
6.5–7.5	Good control	3–5	0.35	2.56	0.01
7.6–8.5	Fair control	6–8	0.58	4.21	<0.001
8.6–9.5	Poor control	9–12	0.75	6.15	<0.001
> 9.5	Very poor control	>13	0.85	8.42	<0.0001

Additionally, imbalances in specific amino acids, such as elevated glutamine and reduced glycine levels, may contribute to the development of diabetic nephropathy. Early identification and appropriate management of aminoaciduria may therefore represent a valuable adjunctive approach for reducing T2DM-related complications.

The mechanistic target of rapamycin (mTOR) pathway is a key regulator of cellular growth, metabolism, and autophagy. BCAAs—particularly leucine—activate the mTOR pathway, which contributes to insulin resistance by downregulating insulin receptor substrate-1 (IRS-1) expression,

enhancing hepatic gluconeogenesis, and impairing glucose uptake in skeletal muscle.^{9–11}

In the present study, serum levels of BCAAs were significantly elevated, along with increased urinary excretion in patients with T2DM ($p < 0.001$), findings that are consistent with those reported by Kolanu et al.¹² Similarly, a study by Bidi et al. examining urinary amino acid excretion patterns in patients with T2DM and healthy controls demonstrated a higher frequency of excretion of phenylalanine, arginine, tyrosine, and tryptophan in diabetic subjects.^{13,14} Our study further demonstrated increased urinary excretion of all 18 amino acids, of which 14 showed statistically significant elevations ($p < 0.001$).

A study conducted in a common population with diabetic kidney disease (DKD) reported significantly reduced plasma histidine levels compared with diabetic individuals without DKD, suggesting the potential role of amino acids as diagnostic biomarkers for renal impairment in T2DM.¹⁵ Previous research has shown that insufficient circulating histidine levels may exacerbate inflammation and oxidative stress in kidney disease. Conversely, dietary histidine supplementation has been shown to ameliorate these pathological processes. The underlying cause of altered histidine levels is thought to involve dysregulation of histidine metabolism. Maintaining optimal histidine levels may therefore be essential for preventing renal complications associated with diabetes.^{16,17} Pharmacological interventions, alongside lifestyle modifications, have demonstrated potential in preventing or delaying the onset of type 2 diabetes mellitus. Individualized pharmacological strategies may help attenuate the adverse effects of amino acid imbalances on cellular signaling pathways, highlighting amino acid metabolic pathways as

Table-7: Comparing the serum levels of specific amino acids between healthy controls and type 2 diabetic subjects.

Amino acids ($\mu\text{mol/L}$)	Controls	Diabetes mellitus	Significance
Glutamic acid	164.86 ± 12.64	226.54 ± 10.96	Significant
Valine	200.32 ± 8.65	332.43 ± 12.82	
Lysine	128.32 ± 4.98	232.42 ± 5.99	
Leucine	48.43 ± 5.73	64.56 ± 7.24	
Alanine	24.64 ± 8.54	18.64 ± 5.42	
Isoleucine	88.64 ± 3.89	186.32 ± 6.98	
Serine	96.42 ± 6.32	94.26 ± 8.24	Not Significant
Tryptophan	62.43 ± 8.24	66.45 ± 9.26	
Phenylalanine	88.98 ± 10.24	92.43 ± 6.56	
Glycine	360.64 ± 32.86	198.86 ± 28.43	Significant
Histidine	88.42 ± 3.98	72.64 ± 2.64	
Tyrosine	124.56 ± 10.26	164.56 ± 10.261	
Aspartic acid	198.32 ± 4.32	197.6 ± 5.54	Not Significant
Threonine	286.56 ± 5.98	198.65 ± 7.28	Significant
Methionine	42.64 ± 5.62	44.32 ± 9.86	Not Significant
Proline	186.32 ± 7.34	184.54 ± 6.54	
Cystine	223.65 ± 3.98	226.3 ± 12.96	
Arginine	178.65 ± 5.98	156.78 ± 8.64	Significant

promising therapeutic targets.¹⁸ Prolonged treatment with metformin has been shown to modulate circulating branched-chain amino acid (BCAA) levels, with effects extending beyond activation of AMP-activated protein kinase (AMPK) and suppression of hepatic gluconeogenesis. Metformin also downregulates the expression of key BCAA catabolic enzymes, including BCAT2 and BCKDHA. Furthermore, combination therapy with glipizide and metformin has been reported to acutely alter circulating levels of BCAAs and aromatic amino acids, reflecting improved glycaemic control. Low-dose metformin therapy has also been shown to correct abnormalities in glucose metabolism, with integrative metabolomics analyses revealing alterations in amino acid profiles, including increased levels of serine, glycine, and glutamate, and decreased levels of aspartate.^{19–22} Additionally, sodium–glucose

cotransporter-2 (SGLT2) inhibitors, such as empagliflozin, have been reported to increase concentrations of branched-chain amino acid metabolites, whereas dipeptidyl peptidase-4 (DPP-4) inhibitors, including sitagliptin, have been shown to reduce plasma valine levels and modify amino acid patterns in both animal models and patients with type 2 diabetes mellitus.²³ Further research is required to elucidate the mechanisms underlying these complex associations and to facilitate the development of novel and effective pharmacological therapies for type 2 diabetes mellitus.

CONCLUSIONS

Assessment of plasma and urinary amino acid profiles is essential for understanding the pathophysiology of type 2 diabetes mellitus. These profiles may aid in identifying individuals at risk, monitoring disease

progression, and evaluating therapeutic responses. Targeting amino acid metabolism offers promising avenues for the development of novel treatment strategies for T2DM. Incorporating amino acid profiling into clinical practice has the potential to enhance patient outcomes.

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